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ClearLLab 10C Panel

Markers and how they are combined

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IN THIS PAPER YOU WILL

Discover the ClearLLab 10C panel: the first 10-color CE-IVD immunophenotyping reagents for assessing lymphoid and myeloid populations

Explore the power of optimizing your L&L immunophenotyping workflow with fewer steps, fewer tubes and more colors

Experience the utility of the ClearLLab 10C panel as an aid in the differential diagnosis of hematologically abnormal patients having or suspected of having certain hematopoietic neoplasms

Introduction

Flow cytometric immunophenotyping remains an indispensable tool for the diagnosis, classification, staging, and monitoring of hematologic neoplasms. There have been significant advances in flow cytometry instrumentation and availability of an expanded range of antibodies and fluorochromes that have improved the ability to identify different normal cell populations and recognize phenotypic aberrancies.

ClearLLab 10C Panels consists of a set of four 10-Color reagents in dry unitized format, composed of antibodies directed against T, B, NK and Myeloid lineage antigens. These panels are designed to include the primary antibodies which are lineage oriented as per the 2006 Bethesda consensus. The combinations are intended to identify the leukocyte subpopulations that express these antigens alone or in specific co-expression patterns, and are designed to work with both normal samples and samples coming from any patient having or suspected of having the following hematopoietic neoplasms: chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). The specific choices and combinations in the ClearLLab 10C Panels are based on the guiding principles of (1) addressing the clinical indications, (2) accounting for all major cell populations present in the specimen, and (3) providing sufficiently comprehensive identification of all major categories of hematopoietic cell populations in both normal and neoplastic states. (1-10)

The normal residual cells act as internal reference standards and can be used to determine whether a marker is brighter or dimmer than expected on a specific cell population. The inclusion of a backbone and redundant markers within the panels makes it easier to track populations between tubes, merge the data and troubleshoot unexpected results. In the panel the backbone of CD45 and CD34 are included in each tube. CD45 is used for consistent gating across all four tubes. CD34 in combination with weak CD45 positivity can be used to confirm the hematopoietic origin of cells. Some other redundant markers have been included in the panels such as CD13 and HLA-DR, in both M1 and M2 tubes. CD10 and CD19 are included in the lymphoid and myeloid tubes. CD19 in the myeloid tube is especially useful as it is aberrantly expressed in AML associated with t(8:21). (2) CD7 is included in the T cell tube as well as in the M1 cell tube to detect aberrant expression of CD7 in AML. CD200 can be useful in the differential diagnosis of B-cell neoplasms in particular atypical CLL vs Mantle Cell Lymphoma and was included in the B cell tube instead of CD23. (11)

Both mature and immature markers are combined in the panels to provide efficiency as well as allowing for the identification of all cells in the tubes. The construction of the panel is centered on developing individual tubes within lineages that can be combined to yield an informative immunophenotype.

	405 nm		488 nm					633 nm		
ClearLLab 10C Panels	PB (1)	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-A700 (2)	APC-A750 (3)
B Cell Tube	CD19	CD45	kappa	lambda	CD10	CD5	CD200	CD34	CD38	CD20
T Cell Tube	CD3	CD45	TCRγδ	CD4	CD2	CD56	CD5	CD34	CD7	CD8
M1 Cell Tube	CD11b	CD45	CD16	CD7	CD10	CD13	CD64	CD34	CD14	HLA-DR
M2 Cell Tube	CD19	CD45	CD15	CD123	CD117	CD13	CD33	CD34	CD38	HLA-DR

(1) Pacific Blue* (2) APC-Alexa Fluor* 700 (3) APC-Alexa Fluor* 750

ClearLLab B-cell Tube (B) kappa, Lambda, CD10, CD5, CD200, CD34, CD38, CD20, CD19, and CD45

This reagent tube is specifically constructed to resolve normal as well as malignant cells arising from the B-cell lineage.

Reagent	Normal distribution of staining	Utility in mature B-cell malignancy	Comments
Kappa/Lambda	Mature B cells.	Immunoglobulin light chain restriction.	
CD10	Immature T cells and B cells, subset of mature T cells and B cells, and neutrophils.	Germinal center–like phenotype: FL, DLBCL, BL. Frequently present in ALL.	
CD5	T cells and minor B-cell subset.	Expression on B cells: CLL, MCL.	
CD200	Endothelial cells and neurons and by B-cells and a subset of T-cells.	CD200 is uniformly expressed in chronic lymphocytic leukemia (CLL) and absent in mantle cell lymphoma (MCL).	
CD34	B-cell and T-cell precursors and myeloblasts.	ALL.	Also AML.
CD38	Precursor B cells (hematogones), normal follicle center B cells, immature and activated T cells, plasma cells (bright intensity), myeloid and monocytic cells, and erythroid precursors.	Bright intensity staining may indicate plasmacytic differentiation.	
CD20	Acquired during maturation of precursor B cells (hematogones). Mature B-lymphoid cells positive. Absent on most BM plasma cells. Minor T-cell subset.	Supports B-cell lineage. Intensity often differs between subtypes: CLL/SLL dim, FL brighter. Aberrant expression on ALL or PCN.	
CD19	All B cells, including lymphoblasts, mature B lymphoid cells, and most plasma cells.	Indicates B-cell lineage. May demonstrate abnormal Intensity in B-cell neoplasms. Usually absent in plasma cell neoplasms.	Aberrant expression on myeloid cells in AML or MDS.
CD45	All B cells (weaker intensity on precursors and plasma cells), all T cells (weaker intensity on precursors).	Useful in distinguishing mature lymphoid neoplasms (bright intensity) from ALL and PCN (weak intensity to negative).	

ClearLLab T-cell Tube (T) TCR γ/δ, CD4, CD2, CD56, CD5, CD34, CD3, CD8, CD7 and CD45

This tube of reagents is specifically constructed to resolve normal as well as malignant cells arising from the T-cell and NK cell lineages.

Reagent	Normal distribution	Utility in mature T- and NK-cell lymphoid neoplasms	Comments
TCR γ/δ	Mature T cells in association with sCD3.	Classification mature T-cell lymphoid neoplasms. May help to identify restricted population.	
CD4	T-cell subset and monocytes/ histiocytes.	Useful in classification of mature T-cell lymphoid neoplasms.	Also may be positive in AML.
CD2	T cells and NK cells.	Indicator of T- or NK-cell lineage.	May be aberrantly expressed in AML.
CD56	NK cells and NK-like T cells.	Indicator of NK differentiation.	Aberrant expression in AML and PCN. Small subset of regenerating myeloid cells demonstrates weak expression.
CD5	T cells and minor B-cell subset.	Indicator of T-cell lineage. May be aberrantly lost or decreased in intensity.	May be aberrantly expressed on B cells.
CD34	B-cell and T-cell precursors and myeloblasts.	ALL.	
CD3	Acquired during maturation of T cells.	Indicator of T-cell lineage. May be aberrantly lost or decreased in intensity.	
CD8	T-cell subset and some NK cells.	Useful in classification of mature T-cell lymphoid neoplasms.	
CD7	T cells and NK cells.	Indicator of T-cell lineage. May be aberrantly lost or decreased in intensity.	May be aberrantly expressed in AML.
CD45	All B cells (weaker intensity on precursors and plasma cells), all T cells (weaker intensity on precursors).	Useful in distinguishing mature lymphoid neoplasms (bright intensity) from ALL and PCN (weak intensity to negative).	

ClearLLab Myeloid Cell Tube (M1) CD16, CD7, CD10, CD13, CD64, CD34, CD14, HLA-DR, CD11b, CD45-

These tubes of reagents are specifically constructed to resolve normal as well as malignant cells arising from the Myeloid cell lineage.

Reagent	Normal distribution	Utility in myeloid and monocytic neoplasms	Comments
CD16	Maturing neutrophilic cells, monocytes and NK cells.	May be aberrantly expressed in AML, MDS, and MPD.	
CD7	T cells and NK cells.	May be aberrantly expressed in AML, MDS, and MPD.	
CD10	Immature T cells and B cells, subset of mature T cells and B cells, and neutrophils.	Frequently present in ALL.	
CD13	Neutrophilic and monocytic cells.	Indicator of neutrophilic and monocytic lineage in acute leukemia. May be aberrantly expressed in AML, MDS, and MPD.	
CD64	Monocytes and intermediate neutrophilic precursors.	Identification of monocytic differentiation. May be aberrantly expressed in AML, MDS, and MPD.	Gained on mature neutrophils.
CD34	B-cell and T-cell precursors and myeloblasts.	Identification and enumeration of blasts.	Not all blasts are CD34 positive.
CD14	Monocytes.	Indicator of monocytic differentiation.	Not a sensitive marker of immature monocytes.
HLA-DR	Myeloblasts, monocytes, all B cells, activated T cells.	Identification of promyelocytes, such as in APL. May be aberrantly expressed in AML, MDS,and MPD.	Non-APL AML may also be negative.
CD11b	Maturing neutrophilic and monocytic cells, some lymphoid cells.	May be aberrantly expressed in AML, MDS, and MPD.	
CD45	All B cells (weaker intensity on precursors and plasma cells), all T cells (weaker intensity on precursors).	Identification of blasts (CD45 gating often with low side scatter).	

ClearLLab Myeloid Cell Tube (M2) CD15, CD123, CD117, CD13, CD33, CD34, CD38, HLA-DR, CD19, CD45-

Reagent	Normal distribution	Utility in myeloid and monocytic neoplasms	Comments
CD15	Maturing neutrophilic cells and monocytes.	May be aberrantly expressed in AML, MDS, and MPD.	
CD123	Monocytes, neutrophils, basophils, megakaryocytes, and plasma cytoid dendritic cells (bright).	Positive in some AML, especially with monocytic differentiation.	
CD117	Immature neutrophilic cells and mast cells.	Identification myeloblasts and mast cells.	May be present in myeloma and some T-cell neoplasms.
CD13	Neutrophilic and monocytic cells.	Indicator of neutrophilic and monocytic lineage in acute leukemia. May be aberrantly expressed in AML, MDS, and MPD.	
CD33	Neutrophilic and monocytic cells.	May be aberrantly expressed in AML, MDS, and MPD	Some normal variability in intensity of expression.
CD34	B-cell and T-cell precursors and myeloblasts.	Identification and enumeration of blasts.	Not all blasts are CD34 positive.
CD38	Precursor B cells hematogones), normal follicle center B cells, immature and activated T cells, plasma cells (bright intensity), myeloid and monocytic cells, and erythroid precursors.	Identification of early bone marrow progenitor cell populations for further evaluation of phenotypic abnormalities.	
HLA-DR	Myeloblasts, monocytes, all B cells, activated T cells.	Identification of promyelocytes, such as in APL. May be aberrantly expressed in AML, MDS,and MPD.	Non-APL AML may also be negative.
CD19	All B cells, including lymphoblasts, mature B	Aberrant expression on myeloid cells in AML or MDS.	
CD45	All B cells (weaker intensity on precursors and plasma cells), all T cells (weaker intensity on precursors).	Identification of blasts (CD45 gating often with low side scatter).	

Conclusion

Flow cytometric immunophenotyping can provide a sensitive tool to aid in the identification of the presence of hematologic malignancy and assist in demonstrating the absence of disease. The ClearLLab 10C Panels are intended for in vitro diagnostic use for qualitative identification of various cell populations by multiparameter immunophenotyping. These reagents are used as an aid in the differential diagnosis of hematologically abnormal patients having or suspected of having the following hematopoietic neoplasms: chronic leukemia, acute leukemia, non-Hodgkin lymphoma, myeloma, myelodysplastic syndrome (MDS), and/or myeloproliferative neoplasms (MPN). Interpretation of the results should be confirmed by a pathologist or equivalent professional in conjunction with other clinical and laboratory findings.

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